was contaminated with DNA of any other cultivars. A negative kit can be constructed with SNP markers more easily than with STS markers since we can select the cultivar for which a DNA fragment will be detected by choosing the 3'-end genotype of a SNP primer. Indeed, we have developed negative kits for each of the dominant 16 cultivars in Niigata prefecture, Japan, by using SNP markers as well as STS markers described in this study [39]. We expect other negative kits could be easily developed with SNP markers.

Ⅴ. Conclusion

We constructed seven multiplex PCR marker sets composed of 15 SNP and six STS markers to discriminate 114 Japanese rice cultivars including the 10 cultivars with the highest production volumes in Japan in 2014 and other major cultivars from each Japanese prefecture. The main findings of this study are summarized as follows;

① Single and multiplex PCR with SNP markers developed in this study were sufficiently reliable and as stable as the use of STS markers under optimized conditions.

② Seven multiplex PCR marker sets composed of 15 SNP and six STS markers could easily discriminate 112 of 114 Japanese cultivars (the exceptions were 'Hinohikari' and 'Morinokumasan') and one foreign rice cultivar.

③ 'Hinohikari' and 'Morinokumasan' were discriminated by an SSR marker, RM3120.

Ⅵ. Summary

The identification of cultivars is important for the protection of intellectual property rights of breeders, for the maintenance of crop prices for farmers, and for precise product information for consumers. In Japan, several PCR methods have been developed for crop discrimination. To distinguish rice cultivars in particular, several types of DNA markers have been used. Multiplex PCR methods were developed previously using STS markers to reduce labor, time, and cost. Although SNPs are the most abundant polymorphisms among cultivars, SNP marker sets for multiplex PCR have not been created for rice discrimination. The present study's results demonstrate that the agarose gel electrophoresis of multiplex PCR with SNP markers was as reliable as STS markers, and 114 Japanese rice cultivars including the 10 most produced cultivars in Japan in 2014 and other major cultivars from each Japanese prefecture were successfully discriminated using 15 SNP markers, six STS markers and one SSR marker.

Acknowledgments

We thank all of the institutes described in Table 1 for providing rice seeds. We also thank Mr. Shinnosuke Ohmori for helpful discussion and encouragement; Mr. Tsuyoshi Kotake, Tokuya Genba, Koushi Yasaki, Susumu Saito, Masanori Ichihashi, Kenichi Koide and Sinobu Yuminamochi, and Ms. Umeko Watanabe, Kyoko Yukawa, Noriko Ichimura and Naomi Uesaka for their excellent technical assistance.

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中央農研研究報告　26：23-37（2016）
Bull. NARO. Agric. Res. Cent.

SNP・STS・SSRマーカーを用いた日本のイネ114品種識別用のマルチプレックスPCRマーカーセット

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摘　要

農業生産物における品種情報の担保は，育成者の権利を保護し，生産者にはブランド品種としての販売価格設定を可能にし，消費者には安心感を与えることに繋がることから，日本では様々な作物について品種識別技術が開発されている．これらの中で，マルチプレックス化されたSTSマーカーを用い，PCRで増幅したDNA断片をアガーロースゲル電気泳動で分離・検出してイネの品種識別を行う技術は信頼性も高く，高価な機材を使用しないため多くの研究施設で利用が可能である．本研究では，我々が開発したSNPマーカーによるマルチプレックスPCRが，STSマーカーと同様に安定性と信頼性が高いことを示した．さらに，15種類のSNPマーカーと6種類のSTSマーカーからなる7組のマルチプレックスPCRマーカーセットを作成し，1種類のSSRマーカーを併せて用いることで，2014年の日本での作付面積上位十位の品種および各都道府県での主要品種を含む日本のイネ114品種を識別可能であることを示した．

平成27年10月23日受付　平成28年3月15日受理
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